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Scaling of the ankle extensor muscle-tendon units and the biomechanical implications for bipedal hopping locomotion in the post-pouch kangaroo *Macropus fuliginosus*

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Abstract

Bipedal hopping is used by macropods including rat-kangaroos, wallabies and kangaroos (superfamily Macropodoidea). Interspecific scaling of the ankle extensor muscle-tendon units in the lower hindlimbs of these hopping bipeds shows that peak tendon stress increases disproportionately with body size.

Consequently, large kangaroos store and recover more strain energy in their tendons, making hopping more efficient, but their tendons are at greater risk of rupture. This is the first intraspecific scaling analysis on the functional morphology of the ankle extensor muscle-tendon units (gastrocnemius, plantaris and flexor digitorum longus) in one of the largest extant species of hopping mammal, the western grey kangaroo *Macropus fuliginosus* (5.8 – 70.5 kg post-pouch body mass). The effective mechanical advantage of the ankle extensors does not vary with post-pouch body mass, scaling with an exponent not significantly different from 0.0. Therefore, larger kangaroos balance rotational moments around the ankle by generating muscle forces proportional to weight-related gravitational forces.

Maximum force is dependent upon the physiological cross-sectional area of the muscle, which we found scales geometrically with a mean exponent of only 0.67, rather than 1.0. Therefore, larger kangaroos are limited in their capacity to oppose large external forces around the ankle, potentially compromising fast or accelerative hopping. The strain energy return capacity of the ankle extensor tendons increases with a mean exponent of ~ 1.0 , which is much shallower than the exponent derived from interspecific analyses of hopping mammals (~ 1.4 to 1.9). Tendon safety factor (ratio of rupture stress to estimated peak hopping stress) is lowest in the gastrocnemius (< 2), and it decreases with body mass with an exponent of -0.15 , extrapolating to a predicted rupture at 160 kg. Extinct giant kangaroos weighing 250 kg could therefore not have engaged in fast hopping using ‘scaled-up’ lower hindlimb morphology of extant western grey kangaroos.

Key words: hopping, kangaroo, muscle, strain, stress, tendon

Introduction

In terrestrial animals gravitational loading increases with increasing body size, imposing a penalty on being big (Schmidt-Nielsen, 1970; McMahon, 1973; Biewener, 2000, 2005; Dick & Clemente, 2017). If terrestrial animals maintain geometric similarity across their body size range, their linear dimensions would scale with body mass (M_b) raised to the one-third exponent ($M_b^{0.33}$), areas to the two-thirds exponent ($M_b^{0.67}$), and volumes with an exponent of 1.0 ($M_b^{1.0}$), assuming tissue densities do not change with body mass (Calder III, 1996). Scaling according to geometric predictions is termed ‘isometry’, scaling with an exponent higher than that expected geometrically is termed ‘hyperallometry’ (positive allometry), and an exponent lower is termed ‘hypoallometry’ (negative allometry). Because the force of gravity acting on body mass is proportional to the ground reaction force (GRF), which is a function of body weight and duty factor (the fraction of time over a complete stride that the limb contacts the ground), the force required of muscle to oppose the GRF must scale with an exponent of ~ 1.0 . However, the maximum force that muscle can generate is proportional to its physiological cross-sectional area, which for isometry would scale with an exponent of only 0.67. Thus, as body mass increases, an increasing mismatch appears between maximum muscle force and gravitational loading (Biewener, 2005; Dick & Clemente, 2017). This mismatch is exacerbated during fast locomotion because of the increase in peak gravitational forces acting through the limbs and inertial forces required to accelerate and decelerate the limbs (Alexander, 2003; Biewener, 2003). So how do large terrestrial animals avoid collapsing while safely supporting their own weight?

Examination of the biomechanics of different-sized quadrupedal mammals reveals three possible solutions to that problem. Firstly, the effective mechanical advantage (EMA) of extensor limb muscles (muscle:external moment arm ratio) increases with body mass with an average exponent of ~ 0.25 in individuals from 0.1 to 300 kg body mass (Biewener, 1989), allowing larger mammals to balance rotational moments at their limb joints using muscle force less than expected for their body size. This hyperallometry is driven partly by an increase in muscle moment arms with increasing body mass and partly by a decrease in the external (GRF) moment arm with increasing body mass. Hence, smaller

species move with more crouched (bent-leg) postures than large species that move with more upright (straight-leg) postures. However, this solution appears to reach an upper limit at ~300 kg body mass, above which the legs are unable to straighten any further (Biewener, 2000, 2005). Secondly, the physiological cross-sectional area (PCSA) of the limb muscles exhibits hyperallometry, scaling with an exponent of ~0.80 rather than the geometric 0.67 (Alexander et al., 1981; Pollock & Shadwick, 1994), which implies that larger individuals can generate maximum muscle forces that are greater than predicted for their body size. This hyperallometry results primarily from a decrease in the relative fascicle length with increasing body mass, while muscle volume scales more-or-less (isometrically) in direct proportion to body mass. Thirdly, the peak gravitational forces that act through the limbs during fast locomotion may scale with modest hypoallometry owing to an increased duty factor or reduced peak locomotor performance of large terrestrial mammals (Biewener, 2000; Alexander, 2003). An increase in duty factor or a decrease in peak locomotor performance with increasing body size would reduce the maximum joint loads experienced during fast locomotion, and hence reduce the maximum muscle forces required to balance rotational moments around the joints of larger individuals compared with smaller ones. This trade-off could become particularly important in mammals exceeding ~300 kg body mass, when postural changes have reached an upper limit.

Like quadrupedal mammals, bipedal hopping mammals must also adapt to the increase in gravitational loading associated with increasing body mass. Examination of the biomechanics of different-sized macropods including rat-kangaroos, wallabies and kangaroos (superfamily Macropodoidea), reveals markedly-different scaling patterns to those described above for the quadrupeds. Firstly, relative peak gravitational forces during fast hopping in macropods appear to increase with a slightly positive exponent (Bennett, 1987), so that larger hopping bipeds possibly produce hindlimb muscle forces even greater, relative to body size, than smaller species, exacerbating their problem of generating enough muscle force to oppose gravitational force. Secondly, the EMA of the lower hindlimb muscles in macropods scales with a relationship much shallower than that of the quadrupeds, and indeed may be independent of body mass (Bennett & Taylor, 1995; McGowan et al., 2008). Thus, at least at the

lower hindlimbs, larger hopping species receive little or no mechanical assistance to cope with their mass, and must balance rotational moments about the ankle and knee joints by generating proportionately more muscle force. Thirdly, the PCSA of the hindlimb muscles in macropods increases with hyperallometry (e.g. exponents of $\sim 0.80 - 1.20$) that is even steeper than observed in the quadrupeds (Bennett & Taylor, 1995; McGowan et al., 2008), presumably compensating for the generally invariant scaling of hindlimb EMA and relative peak gravitational forces during fast hopping.

How the macropods have evolved to deal with the increase in gravitational loading associated with increasing body size has important implications for the ankle extensor muscle-tendon units of the lower hindlimbs. The hyperallometry of muscle area is not matched by a proportional increase in tendon area, so that maximum stress predicted for the tendons increases with body mass, scaling with an exponent of $\sim 0.20 - 0.50$ (Bennett & Taylor, 1995; McGowan et al., 2008). Higher than expected tendon stress in larger hopping bipeds allows them to store and recover relatively more tendon strain energy during hopping compared to smaller hopping species. Indeed, the strain energy return capacity of the ankle extensor tendons in macropods increases with extreme hyperallometry, scaling with an exponent of $\sim 1.4 - 1.9$ (Bennett & Taylor, 1995; McGowan et al., 2008). The steep scaling of tendon strain energy is consistent with physiological measurements that show that the efficiency of hopping increases with body mass (Baudinette, 1989, 1994), culminating in a conspicuous uncoupling of energy expenditure rate from travel speed in larger wallabies and kangaroos (Dawson & Taylor, 1973; Baudinette et al., 1992; Kram & Dawson, 1998). The efficiency of hopping might be further improved, because faster travel speeds are achieved primarily by increasing stride length, rather than stride frequency, which should reduce the energetic cost of limb cycling (Webster & Dawson, 2003; Dawson & Webster, 2010; Dawson, 2012). In any case, it is inevitable that larger hopping species are at greater risk of tendon rupture due to reduced safety margins, especially as forces increase during fast hopping. Indeed, the likelihood of the in-series tendons of the ankle extensors rupturing has been forecast to impose an upper limit of 140 kg on the body mass of hopping macropods (McGowan et al., 2008), mercifully exceeding an earlier estimate of 35 kg (Bennett & Taylor, 1995), which is incompatible with the body size of large extant species (Dawson,

2012). Both estimates also conflict with the evidence that some of the extinct giant kangaroos of the Pleistocene weighed up to 250 kg (Helgen et al., 2006), which has led some authors to propose that these giant kangaroos may have adopted a bipedal striding gait (Janis et al., 2014).

In this study, we set out to determine for the first time, whether the extreme interspecific scaling relationships of macropods also occur intraspecifically. We also seek to bring some clarification to the conflicting estimates of the body mass at which tendon rupture has been predicted. We examine the intraspecific scaling characteristics of the ankle extensor muscle-tendon units in the lower hindlimb of the post-pouch western grey kangaroo *Macropus fuliginosus* (Desmarest, 1817). In particular, we derive intraspecific scaling relationships for effective mechanical advantage, muscle volume, muscle physiological cross-sectional area, tendon volume, and tendon cross-sectional area. Because the ankle extensors function as an energy-recovery system during hopping, we also derive scaling relationships for peak tendon stress, strain energy return capacity, and tendon safety factor.

Methods

Animal collection

The carcasses of 17 post-pouch western grey kangaroos *Macropus fuliginosus* (8 males, 9 females) from young-at-foot through to adult life stages (5.8 – 70.5 kg body mass) were collected during management culls near Adelaide, Australia, under the approval of the University of Adelaide Animal Ethics Committee (S-2011-223). Carcasses were transported to the laboratory and weighed to 0.05 kg on a calibrated strain gauge scale (PCE-CS 300; PCE Instruments, Southampton, UK). The lower hindlimbs were then quickly removed from each carcass, sealed individually in large plastic bags to prevent water loss, and immediately frozen for ~1 – 3 months until day of dissection.

Moment arms

The lower hindlimbs contain three ankle extensor muscle-tendon units that function to balance rotational moments at the ankle joint (Figure 1). These muscle-tendon units are the medial and lateral heads of the gastrocnemius (GAS), the plantaris (PL) and the flexor digitorum longus (FDL) (Hopwood & Butterfield, 1990). The GAS and PL muscle moment arm ($r_{\text{gas\&pl}}$, cm), and the FDL muscle moment arm (r_{fdl} , cm), are the perpendicular distances between the tendon's line of action and the centre of rotation of the ankle joint, respectively. The external moment arm (r_{ext} , cm) is the perpendicular distance between the ground reaction force and the centre of rotation of the ankle joint (Biewener, 1989; Bennett & Taylor, 1995; McGowan et al., 2005; McGowan et al., 2008). For the purpose of this study, the ankle's centre of rotation was estimated by manual rotation of the limbs. Because the GAS and PL tendons follow a very similar path from their respective muscle bellies to the calcaneus, their moment arms at the ankle are virtually identical, and so can be treated as one. The ground reaction force is assumed to act in a vertical direction halfway along the proximal phalanx of digit IV (Bennett & Taylor, 1995; Bennett, 2000). Because moment arms vary slightly over the physiological range of joint angles, we standardised our measurements with the ankle joint held at ~90° (McGowan et al., 2008), which we assume corresponds approximately to the *in vivo* midstance hopping position (when peak forces are expected) across the post-

pouch body mass range of the kangaroos. We used 30-cm rulers to measure moment arms to 0.1 cm (5% maximum rounding error), and calculated the effective mechanical advantage of the GAS and PL ($ema_{gas\&pl}$), and of the FDL (ema_{fdl}), as the quotient of the muscle moment arm and the estimated external (GRF) moment arm.

Muscle-tendon unit morphology

Each muscle-tendon unit was dissected free, and its total length (l_{mtu} ; cm) measured by ruler to 0.1 cm, and its total mass (m_{mtu} ; g) measured to 1 g on a digital balance (QS 16; Sartorius, Göttingen, Germany). The muscle belly was isolated from each muscle-tendon unit, and its length (l_m ; cm) and mass (m_m ; g) recorded to the same precisions. Muscle volume (V_m ; cm³) was calculated as $V_m = m_m \rho_m^{-1}$, where ρ_m is the average mass-density of mammalian muscle [= 1.06 g cm⁻³ (Méndez & Keys, 1960)]. An incision was then made along the length of the muscle, so that it could be parted along its central axis, photographed with a scale, and five measurements made of fascicle length (l_f ; cm), and five measurements made of pennation angle (θ ; radian), using computer graphics software (CorelDRAW 15; Corel Corporation, Ottawa, ON, Canada). The physiological cross-sectional area of each muscle (A_m ; cm²) could then be obtained given, $A_m = V_m \cos\theta / l_f$, where l_f and θ are the average values from each muscle (Sacks & Roy, 1982). Because muscle area varies moderately over the physiological range of fascicle lengths, we standardised our measurements using slack muscle dissected off the bone (McGowan et al., 2008), which we assume corresponds approximately to the *in vivo* midstance hopping position across the post-pouch body mass range of the kangaroos.

The extramuscular tendon (tendon external to the muscle belly) of each muscle-tendon unit was laid straight and its length ($l_{t,ext}$; cm) measured to 0.1 cm, and its mass ($m_{t,ext}$; g) recorded to 0.01 g on a digital balance (1265 MP; Sartorius). The small regions of the PL and FDL tendons that pass behind the calcaneus and ankle, respectively, have low elastic moduli relative to other parts of the tendon (Ker et al., 1986), and were thus removed prior to measurement (McGowan et al., 2008). The intramuscular tendon

(tendon within the muscle belly) could not be measured directly, but its length ($l_{t,int}$; cm) was determined given, $l_{t,int} = l_{mtu} - l_{t,ext} - l_f \cos \theta$. Thus, total tendon length ($l_{t,int+ext}$) was obtained from the sum of $l_{t,int}$ and $l_{t,ext}$. The cross-sectional area of each tendon (A_t ; cm²) was calculated as, $A_t = m_{t,ext} / (\rho_t l_{t,ext})$, where ρ_t is the mass-density of tendon [= 1.12 g cm⁻³ (Ker, 1981)]. The total volume of each tendon (V_t ; cm³) was then calculated given, $V_t = A_t l_{t,int+ext}$. Once again, we assume that our measurements using slack tendon dissected off the bone (McGowan et al., 2008) corresponds approximately to the *in vivo* midstance hopping position across the post-pouch body mass range of the kangaroos.

Tendon stress loading, strain energy return capacity, and safety factor

The muscle and tendon are connected in series, allowing peak tendon stress ($\sigma_{t,max}$; MPa) to be estimated from, $\sigma_{t,max} = \sigma_{m,max} (A_m / A_t)$ (Ker et al., 1988; Pollock & Shadwick, 1994), where $\sigma_{m,max}$ is maximum isometric stress generated by mammalian muscle [= 0.3 MPa (Biewener, 2003)]. Here, we assume that A_m corresponds approximately to the optimal sarcomere length for producing maximum force. The strain energy return capacity of the tendon (U_t ; J) was then estimated according to the equation, $U_t = V_t \left(\frac{\sigma_{t,max}^2}{2E} \right) 0.93$ (Biewener & Baudinette, 1995; McGowan et al., 2008), where E is the elastic modulus of tendon operating over its functional stress range as measured *in vivo* from tammar wallaby [= 1000 MPa (Biewener & Baudinette, 1995)] and 0.93 accounts for 7% energy loss due to mechanical hysteresis (Bennett et al., 1986; Biewener, 2008). Lastly, the safety factor built into the tendon (S_t) was estimated as, $S_t = \sigma_{t,fail} / \sigma_{t,max}$, where $\sigma_{t,fail}$ is the stress under which tendons are known to rupture [= 100 MPa (Bennett et al., 1986)]. Although the earlier interspecific studies on the ankle extensor muscle-tendon units of macropods assumed that $\sigma_{m,max}$, E , $\sigma_{t,fail}$ and hysteresis energy loss are constant with body mass (Bennett & Taylor, 1995; McGowan et al., 2008), we suggest that this may require validation across the intraspecific body mass range of the post-pouch kangaroo, particularly for the young-at-foot, where the muscle-tendon building blocks may not yet be completely mature.

Statistical analyses

Measurements were taken from both the left and right lower hindlimbs and averaged for each individual. Data for males and females were analysed together to obtain a suitable sample size and body mass range for scaling analysis. Scaling relationships were obtained by calculating ordinary least-squares linear regressions of \log_{10} -transformed data with body mass as the independent variable. Ordinary least-squares was used for this study because its assumptions are approximately satisfied and because the error in X is generally lower than the error in Y (Smith, 2009; Kilmer & Rodriguez, 2017). An F-test assessed the statistical significance of the correlation, and the coefficient of determination (r^2) evaluated its strength, for each regression. An ANCOVA tested for significant differences in slope (scaling exponent) and in elevation (scaling coefficient) between regressions with body mass as the covariate (Zar, 1998). Statistical significance was set at 0.05 *a priori* and all analyses were performed using statistical software (GraphPad Prism 6; GraphPad Software, La Jolla, CA, USA). Although statistical analyses were performed on \log_{10} -transformed data, we report the scaling relationships in the more familiar form of a power equation, $y = aM_b^{b \pm 95\% \text{ CI}}$, where y is the parameter of interest, a is the scaling coefficient (elevation), b is the scaling exponent (slope of the log transformed relationship), M_b is body mass in kg, and CI stands for confidence interval. All power equations presented in this paper were derived using averaged values of both legs per individual, and as such, return the mean value for a single leg only.

Results

Moment arms

Across the 12-fold body mass range of post-pouch western grey kangaroos, the muscle moment arms ($r_{\text{gas\&pl}}$ and r_{fdl}) and the external moment arm (r_{ext}) scale with statistically indistinguishable exponents (overall mean exponent = 0.19; Table 1). These exponents are hypoallometric, as evident by the upper 95% confidence limits, which are less than the geometrically proportional exponent of 0.33 predicted for linear dimensions (Table 1). Because the muscle moment arms scale in parallel with the external moment arm, the effective mechanical advantage of the GAS, PL ($ema_{\text{gas\&pl}}$) and of the FDL (ema_{fdl}), does not vary with body mass, and hence scales with exponents that are not significantly different from 0.0 (Table 1). However, there is a significant difference in the elevations, which reflects the ~3.2-fold greater effective mechanical advantage of the GAS and PL relative to the FDL.

Muscle-tendon unit morphology

The muscle volumes (V_m) for the individual muscle-tendon units (GAS, PL and FDL) scale on body mass with statistically indistinguishable exponents (overall mean exponent = 0.86; Table 2). Once again, these exponents are hypoallometric, since the upper 95% confidence limits are less than the geometrically proportional exponent of 1.0 predicted for volumes (Table 2). However, there is a significant difference in the elevations, which arises because the GAS muscle volume is, on average, ~1.3-fold larger than PL muscle volume, and ~2.7-fold larger than FDL muscle volume. In the three muscles, fascicle length (l_f) scales with statistically indistinguishable exponents, with an overall mean hypoallometric exponent of 0.18 (Table 2). Likewise, pennation angle (θ) in the three muscles scales with statistically indistinguishable exponents (Table 2), and remains constant across body mass (scaling exponents ≈ 0.0). The physiological cross-sectional areas of the three muscles (A_m) also scale with statistically indistinguishable exponents with an overall mean isometric exponent of 0.67 (Table 2), exactly as expected for geometric similarity. There is nonetheless a significant difference in the elevations of the

regressions for muscle area, which arises because the GAS muscle area is, on average, ~1.2-fold larger than that of the PL, and ~2.6-fold larger than that of the FDL.

The tendon volumes (V_t) for the individual muscle-tendon units (GAS, PL and FDL) scale on body mass with statistically indistinguishable exponents (overall mean exponent = 0.90; Table 2). Nonetheless, there is a significant difference in the elevations of the regressions for tendon volume, which arises because the shorter GAS tendon is, on average, approximately one-half the volume of the longer PL and FDL tendons. The cross-sectional area of the three tendons (A_t) also scales with statistically indistinguishable exponents with an overall pooled exponent of 0.63 (Table 2), slightly less than the geometrically proportional exponent of 0.67 expected for areas. This result mainly reflects the hypoallometric exponent of 0.55 ± 0.08 derived for the cross-sectional area of the GAS tendon. Consequently, the ratio between the physiological cross-sectional area of the muscle and the cross-sectional area of the tendon (A_m/A_t) increases significantly with body mass in the GAS, scaling with an exponent of 0.15 ± 0.10 , but is independent of body mass in the PL and FDL, scaling with exponents not significantly different from 0.0 (Table 2).

Tendon stress loading, strain energy return capacity, and safety factor

Estimated peak tendon stress ($\sigma_{t,max}$) increases with body mass in the GAS, scaling with an exponent of 0.15 ± 0.10 , but is invariant of body mass in the PL and FDL (Table 3). It is also evident that the estimated peak stress within the GAS and PL tendons is ~3-fold greater than for the FDL tendon (Figure 2). The strain energy return capacity of the tendons (U_t) increase significantly with body mass in the GAS, PL and FDL (Table 3). Nonetheless, the GAS and PL tendons play a much larger role in strain energy storage and recovery compared to the FDL tendon (Figure 3). The estimated safety factor (S_t) of the GAS tendon decreases with body mass, scaling with an exponent of -0.15 ± 0.10 , while the PL and FDL tendon safety factors are independent of body mass (Table 3). For our estimated peak locomotor stresses, the GAS and PL tendons have a modest safety factor in the range of 1.1 to 1.9, whereas the FDL tendon has a much

greater safety factor of ~4.4 (Figure 4). Because we cannot be certain that the muscle-tendon building blocks in the three young-at-foot individuals have reached structural and functional maturity, our assumed values for muscle maximum isometric stress, tendon elastic modulus, tendon rupture point and hysteresis energy loss may require validation, and so we provide a set of revised scaling relationships that exclude these young-at-foot individuals (Table 3). Exclusion of these individuals does not affect these scaling relationships significantly (ANCOVA, $P > 0.05$ for comparisons of slope and elevation), however, the estimated GAS tendon safety factor no longer decreases significantly with body mass (Table 3).

Discussion

Hopping mammals and quadrupedal mammals have evolved differing solutions to manage the increase in gravitational loading associated with increasing body size. Interspecific examination of the biomechanics of different-sized macropods reveal that these hopping bipeds rely almost entirely on the hyperallometry of the PCSA of the limb muscles to support their weight during locomotion (Bennett & Taylor, 1995; McGowan et al., 2008). However, the hyperallometry of the PCSA is not matched by a parallel hyperallometry of tendon cross-sectional area, and because of this mismatch, ankle extensor peak tendon stresses increase with body size, such that larger species of hopping biped operate closer to the point of tendon rupture. Hence, our principal aim was to determine whether the extreme interspecific scaling relationships derived for macropod ankle extensors also are evident at an intraspecific level. We investigated the post-pouch western grey kangaroo, which is among the largest of the extant hopping mammals.

Interspecific scaling of hopping mammals shows that the muscle moment arms of the ankle extensors scale with hyperallometry with an exponent of $\sim 0.43 - 0.46$ (Bennett & Taylor, 1995; McGowan et al., 2008), and that the external moment arm of the GRF appears to scale in parallel with muscle moment arms (Bennett & Taylor, 1995), or potentially with a shallower exponent (McGowan et al., 2008). Consequently, the EMA of the ankle extensors is either independent of body mass, or it increases with a slightly positive exponent. Our intraspecific analysis on post-pouch western grey kangaroos shows that the EMA of the ankle extensors is independent of body mass, but this independence is achieved by the parallel hypoallometry of both muscle and external (GRF) moment arms, which each scale with an overall mean exponent of 0.19 (Table 1). That ankle extensor EMA does not vary significantly with body mass implies that larger kangaroos balance rotational moments around the ankle by generating proportionately greater muscle forces (i.e. muscle forces proportional to weight-related gravitational forces).

Interspecific analyses of hopping mammals show that the PCSA of the ankle extensor muscles increases with very steep hyperallometry, scaling with an exponent of $\sim 0.80 - 1.20$, which is much

steeper than isometry (exponent of 0.67), and is required primarily to compensate for the body mass-independence of muscle EMA across species (Bennett & Taylor, 1995; McGowan et al., 2008). Our intraspecific analysis shows that EMA is independent of body mass, but there is no steep hyperallometry of muscle PCSA. Instead, ankle extensor muscle volume and fascicle length both scale with hypoallometry, with mean exponents of 0.86 and 0.18, respectively, such that muscle PCSA scales with geometric similarity (mean exponent of 0.67; Table 2). Thus, the body mass-independence of muscle EMA, the hypoallometry of the muscle moment arms, and the geometric scaling of muscle PCSA, combine to imply that smaller western grey kangaroos generate relatively greater maximum ankle torques for their size than do larger kangaroos. Smaller western grey kangaroos also have hearts that are relatively larger for their body mass than do larger kangaroos (Snelling et al., 2015a; Snelling et al., 2015b). Relatively larger hearts (for aerobic capacity) and relatively greater ankle torques (for acceleration and hopping speed) of smaller kangaroos may reflect their greater predation risk (Dawson, 2012). Predation pressure is thought to influence the ontogeny of locomotor performance in other species of mammal. For instance, the locomotor performance of the black-tailed jackrabbit *Lepus californicus* is relatively high in the juveniles compared to the adults (Carrier, 1983). It is also possible that the relatively greater ankle torques of smaller kangaroos may compensate for reduced functional performance associated with growth processes (Carrier, 1996). This could be particularly relevant to the young-at-foot, where the muscle and tendon building blocks may not yet have reached structural and functional maturity (Shadwick, 1990).

Interspecific scaling of hopping mammals shows that a mismatch, which increases with body mass, occurs between the PCSA of the muscle and the cross-sectional area of the tendon of the ankle extensors. This mismatch results in the very steep hyperallometry of the predicted maximum tendon stress, scaling with body mass with an exponent of $\sim 0.20 - 0.50$, as well as extreme hyperallometry of the strain energy return capacity of the tendons, scaling with body mass with an exponent of $\sim 1.4 - 1.9$ (Bennett & Taylor, 1995; McGowan et al., 2008). In our intraspecific analysis, the scaling relationships for tendon stress (exponents in the range of -0.06 to 0.15) and strain energy return capacity (0.84 to 1.16) are much shallower than those in the interspecific analyses. Our study shows that the GAS tendon cross-sectional

area scales with moderate hypoallometry with an exponent of 0.55, whereas the PL and FDL tendon cross-sectional areas approximately scale with the expected geometric exponent of 0.67 (Table 2). Because the physiological cross-sectional area of the GAS, PL and FDL muscles scale with the mean geometric exponent of 0.67, the estimated peak stress loading of the GAS tendon increases with body mass, scaling with an exponent of 0.15, whereas peak tendon stresses of the PL and FDL remain independent of body mass (Figure 2). As a consequence of the scaling of the estimated stress, the strain energy return capacity of the GAS tendon scales with a steeper exponent of 1.16, compared to that of the PL and FDL tendons, which scale with shallower exponents of 0.93 and 0.84, respectively (Figure 3). Importantly, it is also evident that the GAS and PL tendons play the major role in energy storage and recovery during locomotion, with the FDL tendon contributing much less (Figure 3). This finding is consistent with the GAS and PL tendons functioning as ‘high-stress’ tendons specialized for elastic energy savings, whereas the FDL tendon functions as a ‘low-stress’ tendon adapted to minimize structural compliance and provide a finer control of limb position (Alexander, 2002; Biewener, 2008).

Although the ‘high-stress’ tendon design allows for considerable energy savings during locomotion (Cavagna et al., 1977; Biewener & Baudinette, 1995), there is a greater risk of rupture due to a much reduced safety factor (Biewener & Bertram, 1991; Biewener, 2008). Consistent with the finding from the larger species examined in the interspecific scaling analyses of hopping mammals (Bennett & Taylor, 1995; McGowan et al., 2008), we found that across the intraspecific body mass range of our post-pouch western grey kangaroos, the GAS and PL tendons have an average safety factor of less than 2 (Figure 4). Maintaining tendon stresses within a narrow safety factor is understandable given that tendons cannot be subjected to stresses greater than their muscles can exert (Ker et al., 1988). Nonetheless, it is notable that the GAS tendon safety factor decreases with body mass in the kangaroos, and in some of the largest individuals it appears that the tendon is only just thick enough to withstand the maximum force transmitted to it from the muscle. Extrapolation of our mean regression line, a procedure not without peril, predicts GAS tendon rupture at 160 kg body mass (Figure 4), which is much larger than that determined in an earlier interspecific scaling analysis of hopping mammals of 35 kg (Bennett & Taylor,

1995), but consistent with a more-recent analysis that predicted rupture at 140 kg (McGowan et al., 2008). Nonetheless, the extinct giant kangaroos of the Pleistocene potentially weighed up to 250 kg (Helgen et al., 2006), and so it seems these giant kangaroos could therefore not have engaged in fast hopping using ‘scaled-up’ lower hindlimb morphology of extant western grey kangaroos. The giant kangaroos either had relatively thicker tendons or they did not reach high speeds during hopping.

In summary, we examined the intraspecific scaling characteristics of the ankle extensor muscle-tendon units of post-pouch western grey kangaroos. Our analysis shows that the ankle extensor EMA does not vary with body mass, and so ankle moments must be balanced by muscle forces proportional to weight-related gravitational forces. During fast hopping, gravitational forces acting through the limbs increase markedly, however maximum muscle force is proportional to the muscle’s PCSA, which we found scales geometrically with an exponent of only 0.67. Thus, smaller kangaroos can generate relatively greater force for their size, whereas larger kangaroos are more limited in the forces that their ankle extensors can generate to support hopping. We also found high levels of peak tendon stress and strain energy return capacity in our western grey kangaroos, but neither of these parameters increases with the very steep hyperallometry evident in interspecific scaling analyses of hopping mammals. Finally, extrapolation of the estimated safety factor for the GAS tendon predicts rupture at approximately 160 kg body mass, rendering tendon rupture unlikely in extant kangaroos, but implying that 250 kg extinct giant kangaroos either had relatively thicker tendons, or did not hop at high speeds.

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Author contributions

All authors contributed significantly to the study.

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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Tables

Table 1. Scaling of the moment arms and the effective mechanical advantage of the muscle-tendon units in the lower hindlimb of the post-pouch western grey kangaroo *Macropus fuliginosus* ($N = 17$ individuals).

	GAS & PL	FDL	external	ANCOVA slope (elevation) [‡]
muscle and external moment arm, r (cm)	$2.49M_b^{0.23 \pm 0.05}$, $r^2 = 0.86$, $P < 0.05$	$0.93M_b^{0.17 \pm 0.09}$, $r^2 = 0.52$, $P < 0.05$	$9.44M_b^{0.17 \pm 0.06}$, $r^2 = 0.70$, $P < 0.05$	$F_{2,45} = 1.14$, $P = 0.33$ ($F_{2,47} = 2235$, $P < 0.05$)
effective mechanical advantage, ema	$0.26M_b^{0.06 \pm 0.10}$, $r^2 = 0.11$, $P = 0.19$	$0.10M_b^{0.00 \pm 0.13}$, $r^2 = 0.00$, $P = 0.96$		$F_{1,30} = 0.57$, $P = 0.46$ ($F_{1,31} = 411$, $P < 0.05$)

Equations are in the form $y = aM_b^{b \pm 95\% \text{ CI}}$ where y is the parameter of interest, a is the scaling coefficient (elevation), b is the scaling exponent (slope of the log transformed relationship), M_b is body mass in kg, and CI stands for confidence interval. Equations were derived from the average value of both lower hindlimbs from each individual. Equations return the mean value for a single leg only. [‡]ANCOVA test for statistical significance between slopes (and elevations if applicable). GAS is gastrocnemius, PL is plantaris and FDL is flexor digitorum longus.

Table 2. Scaling of the morphology of the muscle-tendon units in the lower hindlimb of the post-pouch western grey kangaroo ($N = 17$).

	GAS	PL	FDL	ANCOVA slope (elevation)
muscle-tendon unit mass, m_{mtu} (g)	$13.8M_b^{0.85 \pm 0.05}$, $r^2 = 0.99$, $P < 0.05$	$11.4M_b^{0.86 \pm 0.05}$, $r^2 = 0.99$, $P < 0.05$	$5.38M_b^{0.87 \pm 0.05}$, $r^2 = 0.99$, $P < 0.05$	$F_{2,45} = 0.21$, $P = 0.81$ ($F_{2,47} = 632$, $P < 0.05$)
muscle-tendon unit length, l_{mtu} (cm)	$15.9M_b^{0.30 \pm 0.02}$, $r^2 = 0.98$, $P < 0.05$	$29.3M_b^{0.25 \pm 0.02}$, $r^2 = 0.98$, $P < 0.05$	$28.9M_b^{0.24 \pm 0.02}$, $r^2 = 0.98$, $P < 0.05$	$F_{2,45} = 10.4$, $P < 0.05$ (n/a)
muscle volume, V_m (cm ³)	$12.6M_b^{0.85 \pm 0.06}$, $r^2 = 0.99$, $P < 0.05$	$9.65M_b^{0.86 \pm 0.06}$, $r^2 = 0.99$, $P < 0.05$	$4.25M_b^{0.88 \pm 0.05}$, $r^2 = 0.99$, $P < 0.05$	$F_{2,45} = 0.24$, $P = 0.79$ ($F_{2,47} = 744$, $P < 0.05$)
muscle length, l_m (cm)	$6.89M_b^{0.34 \pm 0.04}$, $r^2 = 0.96$, $P < 0.05$	$7.07M_b^{0.36 \pm 0.06}$, $r^2 = 0.93$, $P < 0.05$	$8.20M_b^{0.33 \pm 0.04}$, $r^2 = 0.95$, $P < 0.05$	$F_{2,45} = 0.82$, $P = 0.45$ ($F_{2,47} = 21.2$, $P < 0.05$)
fascicle length, l_f (cm)	$1.88M_b^{0.13 \pm 0.10}$, $r^2 = 0.35$, $P < 0.05$	$1.36M_b^{0.20 \pm 0.10}$, $r^2 = 0.57$, $P < 0.05$	$1.56M_b^{0.21 \pm 0.09}$, $r^2 = 0.63$, $P < 0.05$	$F_{2,45} = 0.87$, $P = 0.42$ ($F_{2,47} = 6.28$, $P < 0.05$)
pennation angle, θ (deg)	$36.1M_b^{0.01 \pm 0.10}$, $r^2 = 0.01$, $P = 0.75$	$39.5M_b^{0.01 \pm 0.04}$, $r^2 = 0.02$, $P = 0.55$	$25.8M_b^{0.05 \pm 0.09}$, $r^2 = 0.07$, $P = 0.29$	$F_{2,45} = 0.26$, $P = 0.77$ ($F_{2,47} = 35.1$, $P < 0.05$)
muscle physiological cross-sectional area, A_m (cm ²)	$5.43M_b^{0.71 \pm 0.09}$, $r^2 = 0.95$, $P < 0.05$	$5.46M_b^{0.65 \pm 0.09}$, $r^2 = 0.94$, $P < 0.05$	$2.45M_b^{0.65 \pm 0.08}$, $r^2 = 0.95$, $P < 0.05$	$F_{2,45} = 0.56$, $P = 0.58$ ($F_{2,47} = 284$, $P < 0.05$)
tendon volume, V_t (cm ³)	$0.51M_b^{0.86 \pm 0.08}$, $r^2 = 0.97$, $P < 0.05$	$0.93M_b^{0.87 \pm 0.11}$, $r^2 = 0.95$, $P < 0.05$	$0.74M_b^{0.96 \pm 0.09}$, $r^2 = 0.97$, $P < 0.05$	$F_{2,45} = 1.36$, $P = 0.27$ ($F_{2,47} = 128$, $P < 0.05$)
tendon length, $l_{t,int+ext}$ (cm)	$14.5M_b^{0.31 \pm 0.03}$, $r^2 = 0.97$, $P < 0.05$	$28.2M_b^{0.25 \pm 0.02}$, $r^2 = 0.98$, $P < 0.05$	$27.5M_b^{0.25 \pm 0.02}$, $r^2 = 0.98$, $P < 0.05$	$F_{2,45} = 11.6$, $P < 0.05$ (n/a)
tendon cross-sectional area, A_t (cm ²)	$0.035M_b^{0.55 \pm 0.08}$, $r^2 = 0.93$, $P < 0.05$	$0.033M_b^{0.63 \pm 0.11}$, $r^2 = 0.90$, $P < 0.05$	$0.027M_b^{0.71 \pm 0.08}$, $r^2 = 0.96$, $P < 0.05$	$F_{2,45} = 3.17$, $P = 0.05$ ($F_{2,47} = 10.9$, $P < 0.05$)
muscle-to-tendon area ratio, A_m/A_t	$155M_b^{0.15 \pm 0.10}$, $r^2 = 0.40$, $P < 0.05$	$166M_b^{0.03 \pm 0.13}$, $r^2 = 0.01$, $P = 0.68$	$91.1M_b^{-0.06 \pm 0.09}$, $r^2 = 0.12$, $P = 0.18$	$F_{2,45} = 4.20$, $P < 0.05$ (n/a)

See Table 1 for abbreviation, regression and statistical explanation.

Table 3. Scaling of the peak stress loading, strain energy return capacity, and safety factor estimated for the tendons in the lower hindlimb of the post-pouch western grey kangaroo ($N = 17$). Also presented is the revised scaling excluding the three smallest young-at-foot individuals for which the assumed values for muscle maximum isometric stress, tendon elastic modulus, tendon rupture point and hysteresis energy loss may require validation ($N = 14$).

	GAS	PL	FDL	ANCOVA slope (elevation)
peak tendon stress, $\sigma_{t,max}$ (MPa)	$46.6M_b^{0.15 \pm 0.10}$, $r^2 = 0.40$, $P < 0.05$	$49.9M_b^{0.03 \pm 0.13}$, $r^2 = 0.01$, $P = 0.68$	$27.3M_b^{-0.06 \pm 0.09}$, $r^2 = 0.12$, $P = 0.18$	$F_{2,45} = 4.20$, $P < 0.05$ (n/a)
excluding young-at-foot	$53.8M_b^{0.11 \pm 0.17}$, $r^2 = 0.15$, $P = 0.18$	$74.2M_b^{-0.09 \pm 0.21}$, $r^2 = 0.06$, $P = 0.38$	$33.5M_b^{-0.12 \pm 0.15}$, $r^2 = 0.20$, $P = 0.11$	$F_{2,36} = 2.35$, $P = 0.11$ ($F_{2,38} = 196$, $P < 0.05$)
strain energy return capacity, U_t (J)	$0.51M_b^{1.16 \pm 0.18}$, $r^2 = 0.93$, $P < 0.05$	$1.07M_b^{0.93 \pm 0.20}$, $r^2 = 0.87$, $P < 0.05$	$0.26M_b^{0.84 \pm 0.15}$, $r^2 = 0.91$, $P < 0.05$	$F_{2,45} = 4.14$, $P < 0.05$ (n/a)
excluding young-at-foot	$0.65M_b^{1.10 \pm 0.26}$, $r^2 = 0.87$, $P < 0.05$	$1.70M_b^{0.79 \pm 0.31}$, $r^2 = 0.72$, $P < 0.05$	$0.33M_b^{0.77 \pm 0.22}$, $r^2 = 0.83$, $P < 0.05$	$F_{2,36} = 2.25$, $P = 0.12$ ($F_{2,38} = 214$, $P < 0.05$)
tendon safety factor, S_t	$2.14M_b^{-0.15 \pm 0.10}$, $r^2 = 0.40$, $P < 0.05$	$2.00M_b^{-0.03 \pm 0.13}$, $r^2 = 0.01$, $P = 0.68$	$3.66M_b^{0.06 \pm 0.09}$, $r^2 = 0.12$, $P = 0.18$	$F_{2,45} = 4.20$, $P < 0.05$ (n/a)
excluding young-at-foot	$1.86M_b^{-0.11 \pm 0.17}$, $r^2 = 0.15$, $P = 0.18$	$1.35M_b^{0.09 \pm 0.21}$, $r^2 = 0.06$, $P = 0.38$	$2.99M_b^{0.12 \pm 0.15}$, $r^2 = 0.20$, $P = 0.11$	$F_{2,36} = 2.35$, $P = 0.11$ ($F_{2,38} = 196$, $P < 0.05$)

See Table 1 for abbreviation, regression and statistical explanation.

Figures

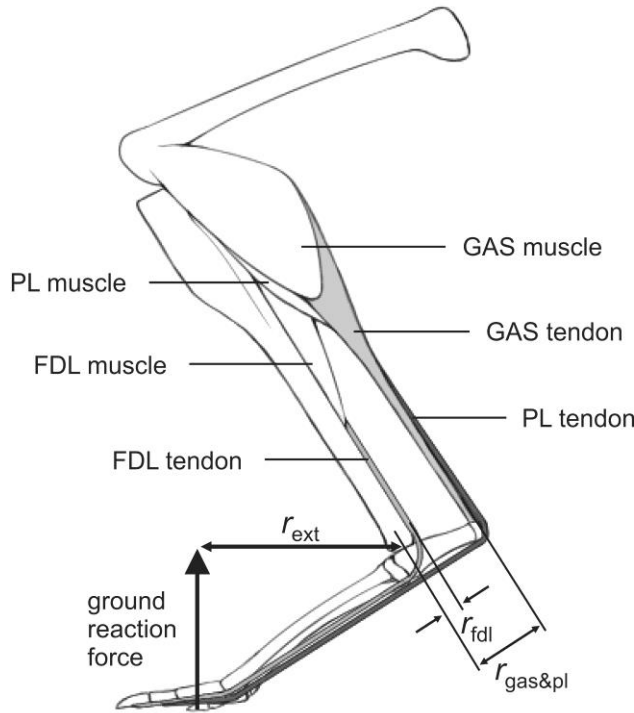


Figure 1. Diagram of the lower hindlimb of the kangaroo in the midstance hopping position. The ankle extensor muscle-tendon units comprise the gastrocnemius (GAS) which inserts at the calcaneus, the plantaris (PL) which passes behind the calcaneus and attaches at the phalanges, and the flexor digitorum longus (FDL) which passes behind the ankle and attaches at the phalanges. Rotation about the ankle joint is balanced by the ankle extensor muscle forces, which operate with the muscle moment arms, $r_{\text{gas\&pl}}$ and r_{fdl} , and the ground reaction force, which operates with the external moment arm, r_{ext} . Adapted from published illustrations (Hopwood & Butterfield, 1990; Bennett & Taylor, 1995; McGowan et al., 2008).

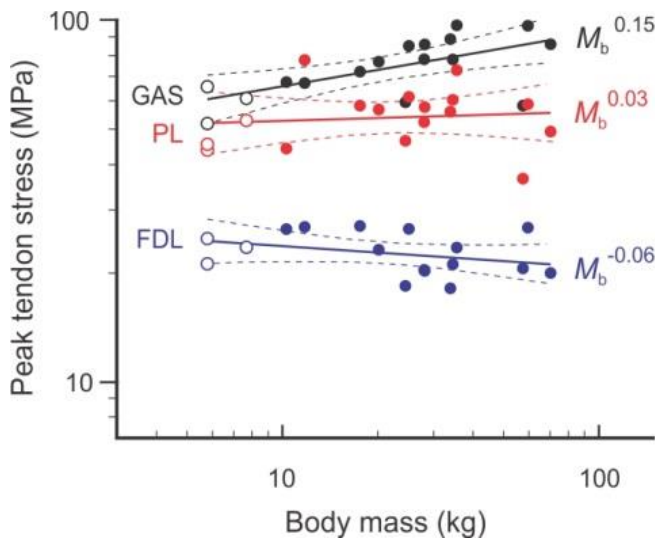


Figure 2. Scaling of the estimated peak stress loading $\sigma_{t,\text{max}}$ on the GAS (black symbols), PL (red) and FDL (blue) tendons in the lower hindlimb of the post-pouch western grey kangaroo *Macropus fuliginosus* ($N = 17$ individuals). Unfilled symbols are young-at-foot for which the assumed value for muscle maximum isometric stress may require validation. See Table 3 for scaling equations and statistics.

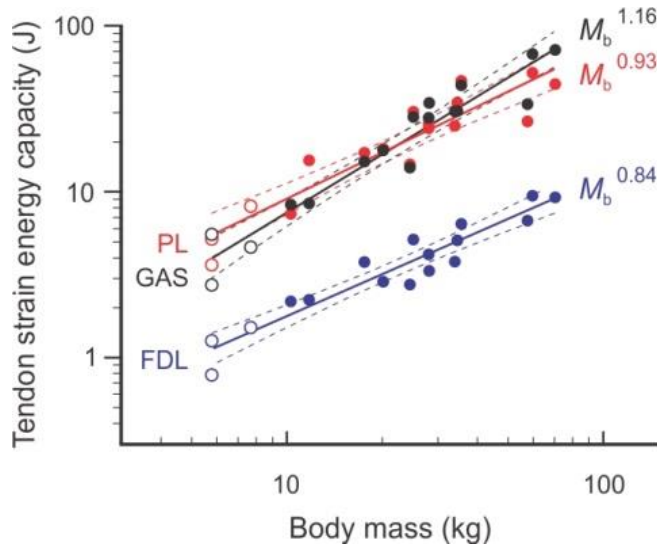


Figure 3. Scaling of the estimated strain energy return capacity U_t of the GAS (black symbols), PL (red) and FDL (blue) tendons in the lower hindlimb of the post-pouch western grey kangaroo ($N = 17$). Unfilled symbols are young-at-foot for which the assumed values for muscle maximum isometric stress, tendon elastic modulus, and hysteresis energy loss may require validation. See Table 3 for scaling equations and statistics.

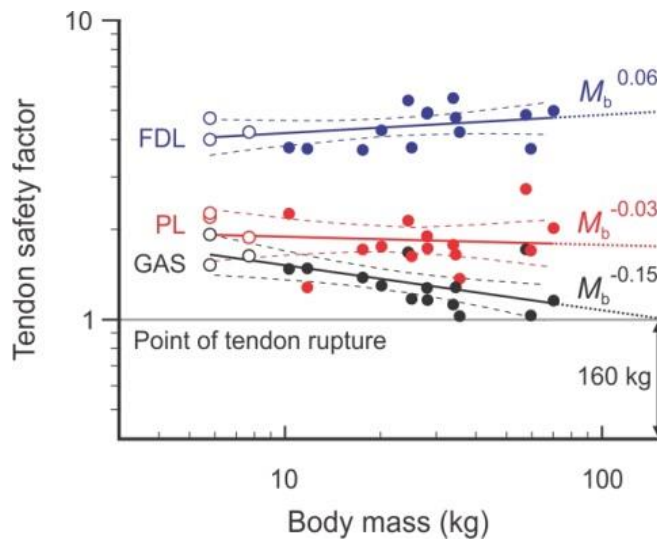


Figure 4. Scaling of the estimated safety factor S_t built into the GAS (black symbols), PL (red) and FDL (blue) tendons in the lower hindlimb of the post-pouch western grey kangaroo ($N = 17$). Unfilled symbols are young-at-foot for which the assumed values for muscle maximum isometric stress and tendon rupture point may require validation. Dotted lines signify extrapolation. The solid grey horizontal line represents a safety factor of 1.0, below which tendons are predicted to fail. See Table 3 for scaling equations and statistics.